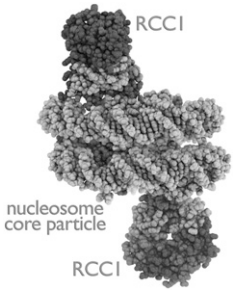
molecular basis for how these chromatin enzymes recognize the 200 kDa nucleosome is largely unknown.



My laboratory investigates how chromatin enzymes interact with the nucleosome. We determined the first crystal structure of a chromatin protein in complex with the nucleosome. Our structure of the 300 kDa RCC1/nucleosome core particle complex at 2.9 Å resolution explains how RCC1's β-propeller domain recognizes the architecture of the nucleosome through a combination of both protein-protein and protein-DNA interactions. Our crystal structure also provides a first atomic view of the nucleosome core particle containing the Widom 601 nucleosome positioning sequence. We find that the 601 DNA forms a 145 bp nucleosome core particle and is thus overwound compared to the human alpha-satellite DNA used in prior nucleosome crystals. I will also discuss the molecular basis for why the Widom 601 DNA sequence is a strong nucleosome positioning sequence.

72-Symp
Unraveling the Higher Order Structure of Chromatin using Single Molecule Force Spectroscopy
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The compaction of eukaryotic DNA into chromatin has been implicated in the regulation of all processes involving DNA. However, the structure of chromatin remains poorly understood. This lack of structural information impedes a functional understanding of chromatin at the molecular level. Here I will discuss recent developments in single molecule force and torque spectroscopy techniques to study this higher order structure.
Using magnetic tweezers and reconstituted designer chromatin fibers, we show that such fibers stretch elastically up to three times their rest length. The stiffness is independent of the presence or absence of linker histones. At 3 pN an overstretching transition occurs that can be attributed to simultaneous rupture of nucleosome-nucleosome interactions and DNA unwrapping.
For quantitative analysis of the compliance of the fibers we use a two-state model in which nucleosomes are stacked, as found in a folded fiber, or unfolded in a beads-on-a-string fiber. All force induced transitions up to 10 pN can be captured in this simple two-state model. Kinetic analysis of the rupture events suggests that stretching of the histone tails precedes the rupture of nucleosome-nucleosome interactions. Changes in extension upon exertion of torsional stress clearly show that the chromatin fibers fold into a left-handed super-helix. Overall, by new single-molecule force spectroscopy techniques and quantitative analysis of the force-extension behavior of single chromatin fibers we resolved a structural and dynamic picture of chromatin folding.

73-Symp
Fluctuations in Chromatin Structure and Gene Expression
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Stochastic models of gene expression assume that promoters randomly transition between transcriptionally active and inactive states, a concept that has proved helpful in understanding expression noise and gene regulation. However, the molecular basis of stochastic promoter switching is unknown. By isolation and electron microscopic analysis of single PHO5 gene molecules from yeast, we show that the PHO5 promoter randomly transitions between alternative nucleosome configurations. Mathematical analysis demonstrates that the probability distribution of alternative promoter nucleosome configurations is obtained by a simple stationary Markov process of nucleosome assembly, disassembly and sliding. Our results provide direct evidence for the stochastic transitioning of a eukaryotic promoter between transcriptionally conducive and inconducive nucleosome configurations, supporting a fundamental proposition of stochastic gene expression models. Combination with fluctuation analysis of single cell gene expression provides novel insights into the role of promoter chromatin structure and dynamics for gene expression and regulation.

Platform: Voltage-gated Na Channels

74-Plat
Crystal Structure of a Pore Only Sodium Channel
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Voltage-gated sodium channels (VGSC) are central to neuromuscular physiology generating the leading phase of action potentials in excitable cells. A variety of marine bacteria produce voltage gated sodium channels that share features with eukaryotic VGSCs and voltage-gated calcium channels (VGCCs). These bacterial channels provide tractable subjects for detailed structural analysis of common features that impact VGSC and VGCC selectivity and gating. We previously reported a structural based design approach in which dissection of the ion conducting module of bacterial VGSCs from the voltage sensor domain created a set of stand-alone 'pore only' channels (Shaya et al. PNAS 2011). These 'pore-only' channels self-assemble as tetramers and form functional, ion selective channels when incorporated into lipid vesicles.
Here, we present the crystal structure of a bacterial 'pore-only' sodium channel. The structure encompasses both the transmembrane and the cytoplasmic parts of the protein and reveals new features that impact channel assembly, gating, and ion permeation.

75-Plat
Access to the Pore of a Voltage-Dependent Na^+ Channel is Controlled by an Intracellular Gate
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Voltage-gated sodium channels (VGSCs) are transmembrane proteins responsible for the initiation and propagation of electrical signals within excitable cells. Upon activation, VGSCs undergo a series of conformational rearrangements transiently opening a Na^+ selective pore. Exactly how Na^+ access the pore remains unclear. It is known that intracellular application of quaternary derivatives of lidocaine, as well as the cytoplasmic tail of the beta-4 subunit produces open channel blockade. Also, mutated residues in the pore-lining S6 helices alter channel gating and disrupt local anesthetic binding. This information supports the notion that a portion of the S6 helices may form an activation gate, much like K^+ channels, which can physically occlude the intracellular mouth of the pore. In order to determine if such a gate exists, we employed the substituted cysteine accessibility method. Cysteine residues were introduced into the domain IV S6 helix and monitored for chemical modification by the positively charged thiol reagent MTSET. Using a fast-inactivation removed Nav1.4 mutant as our background, it was possible to probe the pore without interference from the inactivation gate in inside-out patch clamp recordings. In many sites within the membrane spanning region, cysteine reacted with MTSET only in the open state. More C-terminal positions were modified in both states. This data, in conjunction with the relative location of each residue, suggests that an intracellular activation gate is responsible for granting Na^+ access to the pore.

76-Plat
The Unique Role of the Domain IV Voltage-Sensor in Fast Inactivation
Deborah Capes[1], Manoel Arcisio-Miranda[2], Marcel Goldschen-Ohm[3], Francisco Bezanilla[4], Baron Chanda[3].
[1]Harvard Medical School, Boston, MA, USA, [2]Federal University of Sao Paulo, Sao Paulo, Brazil, [3]University of Wisconsin, Madison, WI, USA, [4]University of Chicago, Chicago, IL, USA.
Unlike the potassium channel, the voltage-sensors of the sodium channel are homologous but not identical. Prior studies have suggested that each voltage-sensor may play a different role in the processes of activation and inactivation. In order to characterize the role of the voltage-sensors in the process of fast inactivation, we neutralized the first three charges in specific voltage-sensors to glutamine. We reasoned that simultaneous neutralization of critical gating charges in a voltage-sensor would be sufficient to functionally impede the affected voltage-sensor and thus allow us to determine how the properties of fast inactivation are altered by removing that particular source of voltage-dependence. Our experiments reveal that activation of the domain (D) IV voltage-sensor allows fast inactivation to occur. Our results provide solid evidence for the unique importance of the DIV voltage-sensor in the process of fast inactivation.

77-Plat
Involvement of Separate Regions of the NAV Channel DIII-IV Linker Revealed through State Dependent Trapping with Genetically Encoded Photochemistry
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Fast inactivation in voltage-gated sodium channels results from rapid and reversible conformational changes in the cytoplasmic linker between domains